Research Program on *Caenorhabditis elegans*

A critical element in the design and implementation of any testing program is the selection of the biological model to use in identifying a potential hazard. For many years, the NTP has primarily relied upon rodent systems to test for the toxicity of environmental agents. The key component driving this decision has been the close similarity between rodents and humans in terms of the critical components governing toxicity such as the physiology, biochemistry and overall structure of the biological systems. Numerous papers have been written comparing these systems, and scientific and regulatory methods have been established to predict response in humans from responses observed in rodents. This work will continue for the foreseeable future.

The recent development of better molecular and computational tools has provided an opportunity for the development of faster screens in genetically modified rodents or in other species. The ability to develop targeted organisms for testing hypotheses is well established in the scientific literature, and has led to substantial insight into the causal mechanisms, therapy and prevention of disease. Independent researchers interested in a particular hypothesis or mechanism they wished to address have done much of this work. There has been very little effort aimed at the translation of these basic research tools into the types of tools necessary for a testing program of the magnitude of the NTP. To begin translating basic toxicological research tools to a more applied testing program, the free-living soil nematode *Caenorhabditis elegans* will be developed as an invertebrate, toxicological model to assess the effects of potential developmental and neurological toxicants on multicellular organisms.

Three issues are combining to make *C. elegans* a practical species for developmental and neurological testing. First, there is a concerted effort by scientists and the public to reduce, replace or refine the use of mammals in laboratory testing. Second, there is a wealth of knowledge available on *C. elegans* including, its complete genomic sequence; an exceptionally detailed database on its cell and developmental biology, which maps the development of every cell from the fertilized egg to the reproductive adult; a map of all of the neuronal pathways, the ability to observe by microscopy all of the somatic cells in living *C. elegans;* the technology to quickly produce of transgenic nematodes; full transcriptome analysis and several studies demonstrating that changes in *C. elegans* following chemical exposure appear to be predictive of developmental shifts and/or neurological damage seen in laboratory studies using rodents. Finally, new tools in robotics, image acquisition and analysis, gene knockout and gene and protein expression measurements make it possible to study complex biological complicated processes in a medium throughput fashion using *C. elegans*.

Several factors of *C. elegans* biology indicate that it can serve as a model in studies of human disease and toxicology. First, a high degree of evolutionary conservation between *C. elegans* and higher organisms is observed in many of the signal transduction and stress-response pathways. For example, homologues of several human proto-oncogenes have been identified in *C. elegans*. Much of our current understanding of the organization of the *ras* signal transduction pathway has been elucidated by genetic and

reverse-genetic analyses of *C. elegans*. In addition, homologues of tumor suppressor genes involved in renal cell carcinoma, hemangioblastoma and breast cancer have been identified. Many of the regulatory processes controlling apoptosis in higher organisms are conserved, and have been elucidated from studies in *C. elegans*. In fact, the 2002 Nobel Prize in Physiology or Medicine was awarded to three *C. elegans* researchers for their research in this field. Because of the evolutionary conservation of many genes and regulatory pathways, *C. elegans* has provided new information into the mechanisms of human diseases including, Menkes and Wilson's diseases, cancer, Alzheimer's disease, neurological disorders and polycystic kidney-disease. In addition, homologues of many of the genes induced in response to toxicant exposure in vertebrates have been identified in *C. elegans*. These include metallothionein, superoxide dismutase, ubiquitin, heat shock proteins 16 and 70, glutathione-S-transferase, p-glycoprotein and catalase. *C. elegans* also contains homologues to many of the vertebrate signal transduction proteins and pathways that have been implicated in regulating cellular responses to toxicant exposure. Because of the evolutionarily conserved nature of signal transduction and stress-response pathways, it is likely that responses elicited in *C. elegans* will be applicable to understanding similar processes in higher organisms, including humans. It has been suggested that "virtually any gene of interest can be studied at the functional level" in *C. elegans*. In addition, recent estimates suggest that >30% of the genes in *C. elegans* will have homologues in humans.

Using contracting mechanisms over the next three years, the NTP intends to develop *C. elegans* as part of a medium throughput screen to assess developmental and neurological changes following environmental exposures. The translation of *C. elegans* from a research tool into a testing tool consists of five main tasks. The overall goal is to evaluate the toxicological response of known or suspected developmental and/or neurological toxicants in *C. elegans* for the purpose of assessing *C. elegans* as a rapid, sensitive, specific, credible and medium throughput non-mammalian model for toxicology studies.

Task 1. Develop methods to measure the toxicity of known and suspected developmental and neurological toxicants in *C. elegans*. This task involves the development of a microscopic imaging system for *C. elegans*, and development of computer and image analysis software for monitoring growth, size, reproduction and movement. It also requires development of a 96 well format for growth, dosing and toxicity testing. This task is expected to take 6 months.

Task 2. Expose *C. elegans* to at least 200 known or suspected developmental and/or neurological toxicants and determine changes in phenotypic characteristics (survival, size, growth, reproduction and movement). The project officer will select the chemicals and pure chemicals will be supplied by the NTP. The contractor will work with NTP staff on bioinformatic aspects of this agreement in terms of developing user-friendly databases, methods of data analysis and the overall presentation of the results. This task is expected to take 6 months.

Task 3. Create and/or obtain GFP-based, stress-responsive transgenic *C. elegans* lines for improving sensitivity and specificity of toxicity screens. A subset of the chemicals tested

in Task 2 will be tested for toxicity in these transgenic lines. The exact transgenic lines to obtain and the chemicals to study will be developed jointly between the contractor and the NTP staff with advice from the NTP Board of Scientific Counselors. This task will also include the development of multi-dimensional (3-D, 4-D) computer imaging software to quantitatively measure the effects of toxicant exposure on nervous system development. This task will continue throughout the remaining part of the contract and will likely take two years.

Task 4. Use *C. elegans* microarray analysis and test a subset of chemicals from Task 2 and Task 3, selected by the NTP, for changes in gene expression. This task will also operate throughout the last two years of the project. Up-regulated genes identified in Task 4 will be used in further generation of transgenic, GFP-based stress-responsive *C. elegans* described in Task 3.

Task 5. Adapt methods for medium throughput analysis to assess the toxicological responses in *C. elegans* in which each gene has been inactivated using RNA interference techniques. Test a subset of chemicals, selected by the project officer, from Tasks 2 and 3 by this method. The NTP and the contractor will jointly choose the chemicals and C. *elegans* lines to use with periodic review by the NTP Board. It is expected that this task will be underway and completed during the last year of this project.

Finally, one additional advantage of this project is the development of robotic tools for medium throughput screening using 96-well plates and computer-assisted imaging. Even if *C. elegans* alone does not completely predict the reproductive and/or developmental toxicity of environmental agents, there are numerous other species that could be studied using the same basic set-up that may prove to be better predictors. The development of methods and skills for medium throughput screening in 96 well plates is necessary for any of these organisms to be used by the NTP.